

**REMARKS**

The Examiner has rejected claim 11 as confusing. Applicants have amended claim 11 to more distinctly claim the subject matter. Applicants believe the rejection is now moot.

The Examiner has rejected claim 6 as improper because of multiple dependencies on a claim that is multiply dependent. Applicants have amended claim 6 to depend on claim 5 only and have added new claim 17, which is similar to claim 6 but depends on claim 3.

**Rejection under 35 U.S.C. §112 first ¶**

GNNPY sequence. The AcuI sequence can be found in new Figures 10-1 – 10-4. GNNPY is present in Motif IV at residue 595 and is shown to be common to BpmI, AcuI, BsgI and ThaIV.

Page 32, lines 13-16. D80A and Phe520. D80A refers to a point mutation in AcuI that results in the inactivation of the cleavage activity of AcuI. Phe520 (F) is the amino acid on AcuI at the fusion junction. The AcuI sequence is now provided in new Figure 10 as requested by the Examiner.

Page 35, lines 1-4: The sequences for BpmI, BsgI, AcuI and ThaIV are provided in Figures 10-1 – 10-4. The sequences FDAIIGNPPY (for BpmI) and FDVILPNPPY (BsgI) as well as FDVIVGNPPY for (AcuI) and FDTILGNPPY (ThaIV) are highlighted in motif IV.

Support for claims 11-16

The Examiner has requested an explanation of support in the specification for Claim 11. Support for the amendments can be found in Figure 1 and also on page 9, line 18 - page 10, line 15 and Example 8. In particular, support for "two-step PCR" in claim 11 can be found in Example 8.

"A method for obtaining a chimeric Type IIG restriction endonuclease containing a Gamma type methylase and having an altered specificity, the restriction endonuclease containing a cleavage domain, a methylase domain and a specificity domain"  
(see page 9, lines 4-28)

"expressing in a host cell, a fusion DNA encoding the chimeric restriction endonuclease, wherein the fusion DNA is formed from a first DNA fragment encoding the cleavage domain and optionally a portion or all of the methylase domain of a first Type IIG restriction endonuclease and a second DNA fragment encoding the specificity domain of a second Type IIG restriction endonuclease"  
(see page 9, lines 17-27, page 11, lines 6-17 )

"(i) selecting primers, for amplifying the first and second DNA fragments by two-step PCR, to form the chimeric Type IIG restriction endonuclease" (Example 8)

"(ii) cleaving the DNA encoding the first and second Type IIG restriction endonuclease with one or more a restriction endonucleases

and ligating the cleaved DNA to form the chimeric Type IIG restriction endonuclease" (see page 11, lines 6-17)

Claims 12 and 13, 15 and 16 are supported by the description in Figure 1 and page 10, line 15 - page 14, line 12.

Claim 17 is the same as claim 6 with a dependency to claim 3 only. The conserved motifs are discussed in Example 4 referencing Malone et al. (1995). The conserved motifs in the Type IIG methylases containing the Gamma type methylase from the Malone reference are provided in new Figure 9.

### Enablement

The specification is enabled for a Type IIG restriction endonuclease with a Gamma type methylase domain as illustrated in Example 8 for BpmI and BsgI for reasons that include the following:

- Each member of the Type IIG endonucleases has cleavage and modification activity that relies on a single specificity domain (target recognition domain or TRD).
  - The modification domain in the claimed Type IIG endonuclease is a group Gamma methylase as shown in Figure 9 which is distinguishable from type Alpha and type Beta by the location of the TRD at the C-terminal end of the protein (Figure 1 and Figure 9).
  - The group Gamma methylase domain is also characterized by highly conserved sequences in motifs X, I-VIII.

- Figure 10 shows how motifs X, I-VIII are present not only in the restriction endonucleases listed by Malone et al. but also in BpmI, AcuI, BsgI and ThaIV.
- In Example 8, BpmI and BsgI are reported to share 35.4% sequence identity (§0095). In addition, BpmI shares 38.6% identity with AcuI and 34.5% sequence identity with ThaIV reflecting the unusual degree of sequence identity in this group of enzymes.
- The principle of two step PCR as described in detail for BpmI and BsgI to form a functional chimeric endonuclease is generally applicable to the claimed class of Type IIG endonucleases specifically those with a Gamma type methylase domain. Figure 10 is provided here to show a sequence comparison of the four restriction enzymes specifically described in the above application. The motifs are shown by boxes and have been annotated. With sequence information, chimeric enzymes can be formed. Significantly, it has been demonstrated in the above application for the first time that the chimeric enzymes formed from two distinct restriction endonucleases are functional.

Applicants demonstrated functionality by showing the activity of the chimeric enzyme *in vivo* by using the SOS induction assay, after confirming the presence of the recombinant DNA in transformed cells by DNA sequencing (see Example 8).

The Examiner has suggested that one of ordinary skill in the art would not know what the chimeric enzymes

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encompassed by the claims would produce. It is believed that this objection is moot in light of the present amendments.

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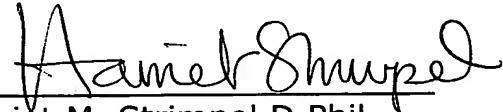
**CONCLUSION**

For the reasons set forth above, Applicants respectfully submit that this case is in condition for immediate allowance. Early and favorable consideration leading to prompt issuance of this Application is earnestly solicited.

Applicants petition for a three-month extension of time and submit check in the amount of \$510 to cover the fees. Please charge any deficiencies to Deposit Account No. 14-0740.

Respectfully submitted,

NEW ENGLAND BIOLABS, INC.



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